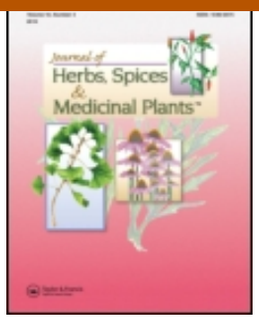

Journal of Herbs, Spices & Medicinal Plants

ISSN: 1049-6475 (Print) 1540-3580 (Online) Journal homepage: http://www.tandfonline.com/loi/whsm20

Evaluation of Antimicrobial and Antioxidant Properties of Pithecolobine Isolated from Albizia saman

Sreerangegowda Thippeswamy, Devihalli Chikkaiah Mohana, Rayasandra Umesh Abhishek & Kiragandur Manjunath

To cite this article: Sreerangegowda Thippeswamy, Devihalli Chikkaiah Mohana, Rayasandra Umesh Abhishek & Kiragandur Manjunath (2015) Evaluation of Antimicrobial and Antioxidant Properties of Pithecolobine Isolated from Albizia saman, Journal of Herbs, Spices & Medicinal Plants, 21:4, 438-446, DOI: 10.1080/10496475.2014.996695

To link to this article: http://dx.doi.org/10.1080/10496475.2014.996695

Journal of Herbs, Spices & Medicinal Plants, 21:438–446, 2015

ISSN: 1049-6475 print/1540-3580 online
DOI: 10.1080/10496475.2014.996695

Evaluation of Antimicrobial and Antioxidant Properties of Pithecolobine Isolated from *Albizia saman*

SREERANGEGOWDA THIPPESWAMY, DEVIHALLI CHIKKAIAH MOHANA, RAYASANDRA UMESH ABHISHEK, and KIRAGANDUR MANJUNATH

Department of Microbiology and Biotechnology, Bangalore University, Jnanabharathi Campus, Bangalore, Karnataka, India

Pithecolobine isolated from alkaloid extract of Albizia saman *showed antimicrobial activity against seven human pathogenic bacteria and two yeasts with minimum inhibitory concentrations (MIC) values of 1.9–125* $\mu g\ mL^{-1}$. *In addition, it also exhibited antioxidant activity with* IC_{50} *value at 250*$\mu g\ mL^{-1}$. *Pithecolobine may be useful as a natural bioactive molecule for developing potent antimicrobial and antioxidant agents.*

KEYWORDS bioactives, DPPH radical scavenging assay, minimum inhibitory concentrations, preparative TLC

INTRODUCTION

The diseases caused by pathogenic microbes pose major problems despite the progress achieved in human health care (7, 16). The continued existence of multidrug-resistant (MDR) pathogenic microbes to currently available drugs has further complicated the treatment of diseases (9, 11). The free radicals generated as by-products in living organisms during metabolic process under oxidative stress conditions play a role in the onset of nearly 150 patho-physiological disorders such as arthritis, diabetes mellitus, inflammatory conditions, cancer, heart, genotoxicity diseases, and early aging (15).

Received March 21, 2014.

Address correspondence to Devihalli Chikkaiah Mohana, Department of Microbiology and Biotechnology, Bangalore University, Jnanabharathi Campus, Mysore Road Street, Bangalore-560 056, Karnataka, India. E-mail: mohanadc@gmail.com

Currently a number of synthetic drugs have been used to prevent diseases and disorders, but many of these synthetic agents are nonspecific and cause serious side effects such as nephrotoxicity, hepatotoxicity, and neurotoxicity, and fail to alleviate the diseases or disorders completely (8, 16, 22). The increasing failures of synthetic chemotherapeutics and serious side effects caused by synthetic drugs have collectively necessitated the need to look for alternative strategies (11, 20). In the past two decades, there has been increasing interest in searching for new and broad-spectrum drugs from natural products as alternatives to synthetic chemicals (14, 21). Alkaloids are important bioactive substances and have been reported for their various bioactivities (1, 6).

Albizia saman (Leguminosae) is rich in alkaloids and globally distributed throughout the tropical regions. The leaves are used in folk remedy for stomach cancer, common cold, diarrhoea, headache, intestinal ailments, sore throat, stomach ache, and wounds (18). Some biological activities of this plant have been reported in aqueous and solvent extracts (3–5, 10, 17, 19, 24), and phytochemical analysis of this species revealed pithecolobine as the main alkaloid (26, 27). Some biological activities have been reported for pithecolobine (2). The present study was done to analyze the antimicrobial and antioxidant activities of pithecolobine.

MATERIALS AND METHODS

Chemicals and Culture Media

Mueller-Hinton agar/broth (MHA/MHB), malt extract-glucose-yeast extract-peptone-agar/broth (MGYPA/MGYPB), dimethyl sulfoxide (DMSO), β-carotene, linoleic acid, neomycin (NM), and fluconazole (FZ) were purchased from Hi-Media, Mumbai, India. All solvents, reagents, ascorbic acid, and iodo-nitro-tetrazolium (INT) were purchased from SRL, Mumbai, India. Microtiter-plates (96-well) were purchased from Axiva, New Delhi, India. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma, Steinheim, Germany. Silica gel 60 F_{254}-coated preparative thin-layer chromatography (TLC) plates were obtained from Merck, Darmstadt, Germany.

Collection of Plant Sample and Identification

Fresh leaves of *A. saman* were collected from southern parts of Karnataka (India) during 2010–2012, authenticated by JCB National Herbarium and voucher specimen (BUB/MB-BT/DCM/JU10/33) deposited in JCB National Herbarium, Indian Institute of Science, Bangalore, India.

Isolation of Bioactive Compounds from Alkaloid Extracts

The leaves were shade-dried, powdered, and used for alkaloid extraction (13). Briefly, 50 g of shade-dried powder was repeatedly extracted with 200 mL of 10% acetic acid in ethanol. The crude alkaloid was precipitated by drop-wise addition of concentrated NH_4OH. The precipitated alkaloid was collected after centrifugation and subjected to isolation of bioactive compound. The bioactive compound was extracted was as described earlier (23). Briefly, the alkaloid extract was first subjected to silica gel column chromatography and eluted with gradient solvent systems of $CHCl_3$ (100%), CH_3OH (100%), and CH_3OH:NH_4OH (1:0.1 and 1:0.5). Twentieth fraction, which showed highest activities, was subjected to preparative TLC for further purification using the solvent system CH_3OH:NH_4OH (1:0.43). The second band (R_f 0.43) showing broad-spectrum activity was collected from TLC. The purity of compound was confirmed using solvent system and subjected to spectral analysis (NMR, ESI-MS, and FT-IR) for structural elucidation.

Antimicrobial Activity

Microbial strains

The pathogenic *Escherichia coli* (NCIM 2065), *Klebsiella pneumoniae* (NCIM 2957), *Proteus vulgaris* (NCIM 2027), *Pseudomonas aeruginosa* (NCIM 5031), *Salmonella typhi* (NCIM 2051), *Staphylococcus aureus* (NCIM 2079), *Streptococcus faecalis* (NCIM 5025), *Candida albicans* (NCIM 3471), and *Cryptococcus neoformans* (NCIM 3541) were obtained from the National Chemical Laboratory, Pune, India. All the tested bacteria and fungi were maintained on MHA and MGYPA, respectively. The bacterial (24 h old) and fungal (48 h old) cultures were used as test organisms.

Disc diffusion method

Briefly, sterile filter paper discs (6 mm in diameter) were individually impregnated with 20 μL of twofold diluted pithecolobine dissolved in DMSO (0.95–1000 μg/disc), then placed onto the pre-inoculated MHA/MGYP plates (inoculum size: 100 μL of microbial suspension containing 10^8 cfu mL^{-1} of bacteria or 10^6 cfu mL^{-1} of fungi) and incubated at 37 °C for bacteria and 30 °C for fungi. DMSO served as negative control, and twofold diluted neomycin (for bacteria) and fluconazole (for fungi) served as positive controls. The zones of inhibition (ZOI) diameters (in mm) were measured (8).

Determination of the MICs and MBCs/MFCs

The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)/minimum fungicidal concentrations (MFCs) of pithecolobine following the standard procedures with some modifications (7, 12). Briefly, 200 μL of twofold serially diluted pithecolobine in MHB or MGYPB (0.95–1000 μg mL^{-1}) was added to the wells of a sterile 96-well microtiter plate and inoculated with 15 μL of a microbial suspension containing 10^8 cfu mL^{-1} of bacteria or 10^6 cfu mL^{-1} of fungi, respectively, and incubated at 37 °C for bacteria (24 h) and 30 °C for fungi (48 h). DMSO served as a negative control, and neomycin and fluconazole were used as positive controls. After incubation, the MIC values of the compounds were detected (12) by the addition of 50 μL of INT (2 mg mL^{-1}). The MBC/MFC values were determined (7). 50 μL of cultured broth (without INT) was radially streaked onto the MHA/MGYPA media and further incubated as described above. The complete absence of growth on the agar surface at the lowest concentration was defined as the MBC/MFC.

Antioxidant Activity

The antioxidant activity of the pithecolobine was determined by DPPH radical scavenging and β-carotene/linoleic acid assays (8). In DPPH assay, twofold dilutions of pithecolobine were made using methanol (ranging from 31–1000 μg mL^{-1}). One mL of each dilution was mixed with 3 mL of freshly prepared methanol solution of DPPH (40 μg mL^{-1}) and incubated for 30 min in the dark at room temperature. The same concentration of ascorbic acid was used as positive control and a methanol solution of DPPH served as negative control. The absorbance of the solutions was recorded using a UV-Vis spectrophotometer at 517 nm, and percent inhibition was calculated as:

$$I\% = \{(A_{control} - A_{sample})/A_{control}\} \times 100$$

where, $A_{control}$ is the absorbance of the control reaction, and A_{sample} is the absorbance of the test samples.

In the β-carotene/linoleic acid assay, 500 μL of pithecolobine (2000 μg in 1000 μL methanol) was added to 2500 μL of β-carotene-linoleic acid emulsion mixture separately, mixed thoroughly, and incubated at 50 °C for 2 h in water bath. Methanol was used as negative control and ascorbic acid was used as positive control. The absorbance was measured at 470 nm using UV-Vis spectrophotometer. The antioxidant activity (Inhibition percentage) was calculated using following formula:

$$I\% = (A_{after\ incubation}/A_{before\ incubation}) \times 100$$

where, $A_{after\ incubation}$ is the absorbance of β-carotene after 2 h, and $A_{before\ incubation}$ is the absorbance of β-carotene at the beginning.

Statistical Analysis

Values were expressed as mean ± SE. Analysis of variance (ANOVA) was performed and the means separated by Tukey's multiple comparison tests ($P < 0.05$) using SPSS 20 (IBM, USA).

RESULTS

Identification of Bioactive Compounds

The IR spectrum of active compounds showed characteristic absorption peaks at (KBr) cm^{-1}: 1646.47, 3353.94, and 2944.94 for strong C=O stretch, N-H stretch, and alkane C-H stretch, respectively. In the positive mode $(M+H)^+$ ESI-MS, the active compound showed molecular ion peak (m/z) at 383.53 corresponding to the molecular formula $C_{22}H_{46}N_4O$ (MW. 382.63). The ^1H NMR analysis of active compound showed characteristic peaks at δ 0.996–1.01 (t, CH_3, J = 8.0 Hz), 1.321–1.402 (m, $4CH_2$, J = 16.8 Hz), 1.605–1.741 (m, $6CH_2$, J = 12.0 Hz), 2.071–2.103 (t, $3CH_2$, J = 13.2 Hz), 2.158–2.196 (t, $3CH_2$, J = 15.2 Hz), 2.691 (s, CH), 2.707–2.750 (q, $4CH_2$, J = 11.6 Hz), 3.229 (s, CH_2), 8.6 (s, N-H), and the ^{13}C NMR analysis showed characteristic peaks at δ 14.92, 15.15, 21.97, 24.11, 24.21, 25.70, 26.91, 27.71, 28.55, 28.66, 29.34, 30.39, 31.05, 33.55, 35.47, 35.80, 38.54, 46.58, 48.86, 49.07, 50.14, 56.29, and 175.00. Further, based on cited literature data (27), the isolated compound was identified as pithecolobine.

Antimicrobial Activity

The antimicrobial activity of pithecolobine was evaluated against human pathogenic microorganisms including seven bacteria and two yeasts by measuring the presence or absence of ZOI, MIC, and MBC/MFC values (Table 1). The bioactive compound pithecolobine showed concentration-dependent bactericidal activity against Gram-positive and Gram-negative bacteria, and fungicidal activity against *C. albicans* and *C. neoformans*. The negative control DMSO did not inhibit any of the microorganisms tested. The maximum ZOI, MIC, and MBC values were observed in the range of 7.6–24.8 mm, 1.9–125 μg mL^{-1}, and 7.8–250 μg mL^{-1}, respectively. Among the bacteria tested, the Gram-positive *S. faecalis* was the most susceptible species, followed by *S. aureus*, whereas the Gram-negative *P. vulgaris* was the most resistant. Similarly, the human pathogenic yeasts *viz., C. albicans* and *C. neoformans* were also inhibited strongly with ZOI, MIC and MFC ranging

TABLE 1 Antimicrobial activity of pithecolobine against selected human pathogenic bacteria and yeasts

Organisms	Pithecolobine			Neomycin/Fluconazole†		
	ZOI[a]	MIC[b]	MBC[c]	ZOI[a]	MIC[b]	MBC[c]
Escherichia coli	15.3 ± 0.3	15.6	62.5	16.8 ± 0.2	3.9	7.8
Klebsiella pneumoniae	15.5 ± 0.3	15.6	31.2	17.1 ± 0.2	1.9	3.9
Proteus vulgaris	7.6 ± 0.2	125	250	20.3 ± 0.3	1.9	3.9
Pseudomonas aeruginosa	13.5 ± 0.3	31.2	62.5	16.6 ± 0.2	1.9	7.8
Salmonella typhi	15.8 ± 0.4	15.6	62.5	19.5 ± 0.3	1.9	3.9
Staphylococcus aureus	19.3 ± 0.3	3.9	15.6	19.5 ± 0.4	0.95	3.9
Streptococcus faecalis	24.8 ± 0.2	1.9	7.8	27.5 ± 0.3	0.95	1.9
Candida albicans	22.3 ± 0.3	7.8	31.2	33.3 ± 0.4*	31.2	>1000
Cryptococcus neoformans	20.1 ± 0.2	15.6	31.2	35.7 ± 0.3*	15.6	500

[a]ZOIs at 0.25mg/disc (mm); [b]MICs (μg mL^{-1}), [c]MBCs/MFCs (μg mL^{-1}).
†Neomycin was used as positive control for bacteria and fluconazole for fungi.
*Fungistatic zone.

from 20.1-22.3mm, 7.8-15.6 μg mL^{-1} and 31.2 μg mL^{-1}, respectively. The antibacterial and antifungal activities of the synthetic antibacterial (neomycin) and antifungal (fluconazole) agents were also determined and compared with the MIC and MBC/MFC values of pithecolobine. Increasing order of inhibitory activity against bacteria was pithecolobine < neomycin and fungi was fluconazole < pithecolobine.

Antioxidant Activity

In the DPPH radical scavenging assay, the IC_{50} value for the pithecolobine was 250 μg mL^{-1} compared to 30 μg mL^{-1} for ascorbic acid. Using the β-carotene/linoleic acid assay, the percent inhibition of β-carotene/linoleic bleaching for the pithecolobine was at 70.4% compared to 93% for ascorbic acid.

DISCUSSION

The increasing documentation of possible adverse effects of some synthetic antimicrobial and antioxidant agents on human health, as well as the continued existence of multidrug-resistant (MDR) human pathogenic bacteria and fungi to currently available antibiotics, lead to the necessity of finding new biomolecules from natural sources for drug discovery and development (9, 11, 14). The identification of bioactive compounds from plants is one of the promising and eco-friendly alternative strategies for the discovery of new drugs (20). Alkaloids are important bioactive substances, and are reported to

have antimicrobial and antioxidant properties (1). *A. saman*, which is globally distributed throughout the tropical regions, is rich in alkaloids. While leaves of *A. saman* are used as folk remedy for diseases, antimicrobial activity reports exist of crude aqueous and solvent extracts of *A. saman* against some human and plant pathogenic bacteria and fungi (2, 5, 17–19, 24). Methanol extracts of *Samanea saman* demonstrated cytotoxic and antioxidant activities in other studies (4, 5).

A wide range biological activities of *A. saman* and pithecolobine have been reported previously (23, 26, 27). Pithecolobine isolated from *S. saman* demonstrated antimicrobial activity against *B. subtilis* and plant pathogenic molds (2). Another report showed antifungal antimycotoxigenic activities of pithecolobine against storage molds (23).

ACKNOWLEDGEMENT

The authors wish to thank the Indian Institute of Science, Bangalore, for providing NMR, FT-IR, and mass spectrometric analysis and spectral interpretation.

FUNDING

This work was financially supported by the Department of Science and Technology, New Delhi, and the University Grant Commission, New Delhi, India.

REFERENCES

1. Ahmad, I., F. Aqil, and M. Owais. 2006. *Modern Phytomedicine—Turning Medicinal Plants into Drugs*. Wiley-VCH Verlag GmbH and Co KGaA, Weinheim, Germany.
2. Ajam S. M. S., B. Salleh, S. Al-khalil, and S. F. Sulaiman. 2012. Antimicrobial activity of spermine alkaloids from *Samanea saman* against microbes associated with sick buildings. *Int. Conf. Environ. Chem. Biol*. 49:150–155.
3. Arulpriya P., P. Lalitha, and S. Hemalatha. 2010. *In vitro* antioxidant testing of the extracts of *Samanea saman* (Jacq.) Merr. *Der Chem. Sinica*. 1:73–79.
4. Arumugam, S., S. V. Selvaraj, S. Velayutham, S. K. Natesan, and K. Palaniswamy. 2011. Evaluation of anti-ulcer activity of *Samanea saman* (Jacq.) Merr. bark on ethanol and stress induced gastric lesions in albino rats. *Indian J. Pharmocol*. 43:586–590.
5. Azhar, I., M. M. Hasan, F. Mazhar, and M. S. Ali. 2009. Some biological evaluations on *Samanea saman*. *Pak. J. Pharmacol*. 26:47–53.

6. Deng, Y., Y. Yu, H. Luo, M. Zhang, X. Qin, and L. Li. 2011. Antimicrobial activity of extract and two alkaloids from traditional Chinese medicinal plant *Stephania dielsiana*. *Food Chem*. 124:1556–1560.
7. Dung, N. T., J. M. Kim, and S. C. Kang. 2008. Chemical composition antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr. and Perry buds. *Food Chem. Toxicol*. 46:3632–3639.
8. Ebrahimabadi, A. H., E. H. Ebrahimabadi, Z. D. Bidgoli, F. J. Kashi, A. Mazoochi, and H. Batooli. 2010. Composition and antioxidant and antimicrobial activity of the essential oil and extracts of *Stachys inflata* Benth. from Iran. *Food Chem*. 119:452–458.
9. Fabri, R. L., M. S. Nogueira, F. G. Braga, E. S. Coimbra, and E. Scio. 2009. *Mitracarpus frigidus* aerial parts exhibited potent antimicrobial antileishmanial and antioxidant effects. *Bioresour. Technol*. 100:428–433.
10. Ferdous, A, M. Z. Imam, and T. Ahmed. 2010. Antioxidant antimicrobial and cytotoxic activities of *Samanea saman* (Jacq.) Merr. *Stamford Journal of Pharmaceutical Sciences*. 3:11–17.
11. Gibbons, S. 2005. Plants as a source of bacterial resistance modulators and anti-infective agents. *Phytochem. Rev*. 4:63–78.
12. Hajji, M., O. Masmoudi, N. Souissi, Y. Triki, S. Kammoun, and M. Nasri. 2010. Chemical composition angiotensin I-converting enzyme (ACE) inhibitory antioxidant and antimicrobial activities of the essential oil from *Periploca laevigata* root barks. *Food Chem*. 121:724–731.
13. Harborne, J. B. 1998. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. Chapan and Hall Publications, London.
14. Ji, H. F., X. J. Li, and H. Y. Zhang, 2009. Natural products and drug discovery. *EMBO Rep*. 10:194–200.
15. Kalim, M. D., D. Bhattacharyya, A. Banerjee, and S. Chattopadhyay. 2010. Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine. *BMC Complement. Altern. Med*. 10:77.
16. Kumaran, A., and R. J. Karunakaran. 2007. Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. *Food Chem*. 100: 356–361.
17. Nnamdi, L. O., C. C. E. Anthony, O. U. Pius, and M. E. Paul. 2010. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* (Fabaceae or Mimosaceae) pods. *Afr. J. Pure Appl. Chem*. 4:206–212.
18. Prasad, R. N., S. Viswanathan, J. R. Devi, V. Nayak, V. C. Swetha, B. R. Aechana, N. Parathasarathy and J. Rajkumar, 2010. Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*. *J. Med. Plants Res*. 2:268–270.
19. Raghavendra, M. P., S. Satish, and K. A. Raveesha. 2008. *In vitro* antibacterial potential of alkaloids of *Samanea saman* (Jacq.) Merr. against *Xanthomonas* and human pathogenic bacteria. *World J. Agr. Sci*. 4:100–105.
20. Reza, S. M. A., A. Rahman, Y. Ahmed, and S. C. Kang. 2010. Inhibition of plant pathogens *in vitro* and *in vivo* with essential oil and organic extracts of *Cestrum nocturnum* L. *Pestic. Biochem. Physiol*. 96:86–92.

21. Riley, M. A., S. M. Robinson, C. M. Roy, M. Dennis, V. Liu, and R. L. Dorit. 2012. Resistance is futile: The bacteriocin model for addressing the antibiotic resistance challenge. *Biochem. Soc. Trans*. 40:1438–1442.
22. Sanglard, D., and F. C. Odds. 2002. Resistance of *Candida* species to antifungal agents: Molecular mechanisms and clinical consequences. *Lancet Infect. Dis*. 2:73–85.
23. Thippeswamy, S., D. C. Mohana, R. U. Abhishek, and K. Manjunath. 2013. Efficacy of bioactive compounds isolated from *Albizia amara* and *Albizia saman* as source of antifungal and antiaflatoxigenic agents. *J. Verbraucherschutz Lebensmittelsicherh*. 8:297–395.
24. Thippeswamy, S., P. Praveen, D. C. Mohana, and K. Manjunath. 2011. Antimicrobial evaluation and phytochemical analysis of known medicinal plant *Samanea saman* (Jacq.) Merr. against some human and plant pathogenic bacteria and fungi. *Int. J. Pharm. Bio. Sci*. 2:443–452.
25. Thippeswamy, S., D. C. Mohana, R. U. Abhishek, and K. Manjunath. 2014. Inhibitory effect of alkaloids of *Albizia amara* and *Albizia saman* on growth and fumonisin B_1 production by *Fusarium verticillioides*. *Int. Food Res. J*. 21(3):947–952.
26. Wiesner, K., D. M. MacDonald, Z. Valenta, and R. Armstrong. 1952. Pithecolobine the alkaloid of *Pithecolobium saman* Benth. I. *Can. J. Chem*. 30:761–772.
27. Wiesner, K., Z. Valenta, D. E. Orr, V. Liede, and G. Kohan. 1968. Structure of Pithecolobine II. *Can. J. Chem*. 46:1881–1886.